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(21) International Application Number: PCT/US96/12371 (22) International Filing Date: 24 July 1996 (24.07.96) (30) Priority Data: 08/522,726 1 September 1995 (01.09.95) US (71) Applicant: THE SALK INSTITUTE FOR BIOLOGICAL STUDIES [US/US]; 10010 North Torrey Pines Road, La Jolla, CA 92037 (US). (72) Inventors: EVANS, Ronald, M.; 1471 Cottontail Lane, La Jolla, CA 92037 (US). CHEN, J., Don; 7405 Charmant Drive #1925, San Diego, CA 92122 (US). (74) Agent: REITER, Stephen, E.; Pretty, Schroeder, Brueggemann & Clark, Suite 2000, 444 South Flower Street, Los Angeles, CA 90071 (US).		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: TRANSCRIPTIONAL CO-REPRESSOR THAT INTERACTS WITH NUCLEAR HORMONE RECEPTORS AND USES THEREFOR (57) Abstract In accordance with the present invention, there are provided novel receptor interacting factors, referred to herein as "SMRT", i.e., a Silencing Mediator (co-repressor) for Retinoic Acid Receptor (RAR) and Thyroid hormone Receptor (TR). SMRT is a novel protein whose association with RAR and TR both in solution and on DNA response elements is destabilized by ligand. The interaction of SMRT with mutant receptors correlates with the transcriptional silencing activities of receptors. <i>in vivo</i> , SMRT functions as a potent co-repressor. A GAL4 DNA binding domain (DBD) fusion of SMRT behaves as a strong repressor of a GAL4-dependent reporter. Together, these data identify a novel class of cofactor which is believed to represent an important mediator of hormone action.		

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Transcriptional Co-repressor that
Interacts with Nuclear Hormone Receptors
and Uses Therefor

FIELD OF THE INVENTION

The present invention relates to intracellular receptors, methods for the modulation thereof, and methods for the identification of novel ligands therefor. In a particular aspect, the present invention relates to methods for the identification of compounds which function as ligands (or ligand precursors) for intracellular receptors. In another aspect, the present invention relates to novel chimeric constructs and uses therefor.

10

BACKGROUND OF THE INVENTION

A central problem in eukaryotic molecular biology continues to be the elucidation of molecules and mechanisms that mediate specific gene regulation. As part of the scientific attack on this problem, a great deal of work has been done in efforts to identify ligands (i.e., exogenous inducers) which are capable of mediating specific gene regulation. Additional work has been done in efforts to identify other molecules involved in specific gene regulation.

20

Although much remains to be learned about the specifics of gene regulation, it is known that ligands modulate gene transcription by acting in concert with intracellular components, including intracellular receptors and discrete DNA sequences known as hormone response elements (HREs).

25

The identification of compounds which directly or indirectly interact with intracellular receptors, and thereby affect transcription of hormone-responsive genes,

would be of significant value, e.g., for therapeutic applications.

Transcriptional silencing mediated by nuclear receptors plays an important role in development, cell differentiation, and is directly linked to the oncogenic activity of v-erbA. The mechanism underlying this effect is unknown but is one key to understanding the molecular basis of hormone action. Accordingly, the identification of components involved in transcriptional silencing would represent a great advance in current understanding of mechanisms that mediate specific gene regulation.

Other information helpful in the understanding and practice of the present invention can be found in commonly assigned United States Patent Nos. 5,071,773 and 4,981,784; and United States Patent Application Nos. 325,240, filed March 17, 1989; 370,407, filed June 22, 1989; and 438,757, filed November 16, 1989, all of which are hereby incorporated herein by reference in their entirety.

20

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have discovered a novel receptor interacting factor, referred to herein as "SMRT", i.e., a silencing mediator (co-repressor) for retinoic acid receptor (RAR) and thyroid hormone receptor (TR). SMRT is a novel protein whose association with RAR and TR both in solution and on DNA response elements is destabilized by ligand. The interaction of SMRT with mutant receptors correlates with the transcriptional silencing activities of receptors.

30

In vivo, SMRT functions as a potent co-repressor. A GAL4 DNA binding domain (DBD) fusion of SMRT behaves as a frank repressor of a GAL4-dependent reporter. Together,

these data identify a novel class of cofactor which is believed to represent an important mediator of hormone action.

BRIEF DESCRIPTION OF THE FIGURES

5 Figure 1 shows the quantitation by phosphoimager of a dose-dependent dissociation of SMRT from RAR or TR by all-trans retinoic acid (atRA) or thyroid hormone (triiodothyronine or T3).

10 Figure 2 presents amino acid (aa) sequences of SMRT (Genbank accession number XXXXX). The aa sequence presented in parentheses (i.e., residues 1330-1376) is an alternatively spliced insert which is not present in the original two-hybrid clone (C-SMRT, aa 981 to C-terminal end). The proline-rich N-terminal domain (aa 1-160) and
15 the glutamine-rich region (aa 1061-1132), as well as the ERDR and SG regions, are also indicated. The C-terminal region of SMRT (aa 1201 to C-terminal end) shows 48% aa identity to RIP13 (Seol et al., *Molecular Endocrinology* 9:72-85 (1995)). The rest of the sequence of RIP13 shows
20 22% aa identity to SMRT (aa 819-1200).

 Figure 3 illustrates mediation of the silencing effect of hRAR α and hTR β by SMRT *in vivo*.

 Figure 3(A) illustrates that v-erbA reverses the silencing effect of GAL-RAR (GAL4 DBD-hRAR α 156-462) while
25 SMRT restores the silencing effect.

 Figure 3(B) illustrates that the RAR403 truncation mutant reverses the silencing effect of GAL-TR (GAL4 DBD-hTR β 173-456) while SMRT restores the silencing effect.

Figure 3(C) illustrates that v-erba and full length SMRT or C-SMRT have no effect on GAL-VP16 activity.

Figure 3(D) illustrates that a GAL4 DBD fusion of full length SMRT suppresses the thymidine kinase basal promoter activity containing four GAL4 binding sites. The fold of repression was calculated by dividing the normalized luciferase activity transfected with the GAL4 DBD alone by those transfected with indicated amount of GAL DBD fusion constructs.

10 DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided co-suppressors of steroid/thyroid hormone receptor activity, said co-suppressors having a structure and function characteristic of the silencing mediator for retinoic acid receptor and thyroid hormone receptor.

Co-suppressors contemplated by the present invention have substantially the same sequence as residues 1-1329 plus 1376-1495, as set forth in SEQ ID NO:1, optionally further comprising the amino acid residues set forth in SEQ ID NO:2 (i. e., residues 1330-1375 of SEQ ID NO:1).

The phrase "substantially the same" is used herein in reference to the nucleotide sequence of DNA, the ribonucleotide sequence of RNA, or the amino acid sequence of protein, that have slight and non-consequential sequence variations from the actual sequences disclosed herein. Species that are substantially the same are considered to be equivalent to the disclosed sequences and as such are within the scope of the appended claims. In this regard, "slight and non-consequential sequence variations" mean that sequences that are substantially the same as the DNA, RNA, or proteins disclosed and claimed herein are

functionally equivalent to the sequences disclosed and claimed herein. Functionally equivalent sequences will function in substantially the same manner to produce substantially the same compositions as the nucleic acid and amino acid compositions disclosed and claimed herein. In particular, functionally equivalent DNAs encode proteins that are the same as those disclosed herein or that have conservative amino acid variations, such as substitution of a non-polar residue for another non-polar residue or a charged residue for a similarly charged residue. These changes include those recognized by those of skill in the art as those that do not substantially alter the tertiary structure of the protein.

In accordance with another aspect of the present invention, there are provided antibodies raised against the above-described co-suppressor. Such antibodies can be employed for studying tissue localization of invention co-repressor, the structure of functional domains, the purification of receptors, as well as in diagnostic applications, therapeutic applications, and the like. Preferably, for therapeutic applications, the antibodies employed will be monoclonal antibodies.

The above-described antibodies can be prepared employing standard techniques, as are well known to those of skill in the art, using the invention co-repressor or portions thereof as antigens for antibody production. Both anti-peptide and anti-fusion protein antibodies can be used [see, for example, Bahouth et al. (1991) Trends Pharmacol Sci. vol. 12:338-343; Current Protocols in Molecular Biology (Ausubel et al., eds.) John Wiley and Sons, New York (1989)]. Factors to consider in selecting portions of invention co-repressor for use as immunogen (as either a synthetic peptide or a recombinantly produced bacterial fusion protein) include antigenicity, accessibility (i.e., where the selected portion is derived from, e.g., the

ligand binding domain, DNA binding domain, dimerization domain, and the like), uniqueness of the particular portion selected (relative to known receptors and co-suppressors therefor), and the like.

5 In accordance with yet another aspect of the present invention, there are provided methods to block the repressing effect of invention co-suppressors, said method comprising administering an effective amount of an antibody as described herein. Alternatively, a silencing domain of
10 a nuclear receptor can be employed. Those of skill in the art can readily determine suitable methods for administering said antibodies, and suitable quantities for administration, which will vary depending on numerous factors, such as the indication being treated, the
15 condition of the subject, and the like.

 In accordance with a still further aspect of the invention, there are provided isolated polynucleic acids encoding the above-described co-suppressor. In addition, there are also provided vectors containing the above-
20 described polynucleic acid.

 In accordance with a still further aspect of the present invention, there are provided complexes comprising the above-described co-suppressor and a homodimeric or heterodimeric member of the steroid/thyroid hormone
25 superfamily of receptors, wherein said member contains a silencing domain which represses basal level promoter activity of target genes. Homodimeric or heterodimeric members of the steroid/thyroid hormone superfamily of receptors contemplated for use herein include thyroid
30 hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, retinoic acid receptor-retinoid X receptor heterodimer, retinoid X receptor homodimer, and the like.

The above-described complexes optionally further comprise a response element for the member of the steroid/thyroid hormone superfamily of receptors. Such response elements are well known in the art. Thus, for example, RAR response elements are composed of at least one direct repeat of two or more half sites separated by a spacer of five nucleotides. The spacer nucleotides can independently be selected from any one of A, C, G or T. Each half site of response elements contemplated for use in the practice of the invention comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-. Response elements employed in the practice of the present invention can optionally be preceded by N_x, wherein x falls in the range of 0 up to 5.

Similarly, TR response elements can be composed of the same half site repeats, with a spacer of four nucleotides. Alternatively, palindromic constructs as have been described in the art are also functional as TR response elements.

The above-described co-repressor/dimeric receptor complexes can be dissociated by contacting complex with a ligand for the member of the steroid/thyroid hormone superfamily of receptors.

As employed herein, the term "ligand (or ligand precursor)" for a member of the steroid/thyroid hormone

superfamily of receptors" (i.e., intracellular receptor) refers to a substance or compound which, in its unmodified form (or after conversion to its "active" form), inside a cell, binds to receptor protein, thereby creating a
5 ligand/receptor complex, which in turn can activate an appropriate hormone response element. A ligand therefore is a compound which acts to modulate gene transcription for a gene maintained under the control of a hormone response element, and includes compounds such as hormones, growth
10 substances, non-hormone compounds that modulate growth, and the like. Ligands include steroid or steroid-like hormone, retinoids, thyroid hormones, pharmaceutically active compounds, and the like. Individual ligands may have the ability to bind to multiple receptors.

15 Accordingly, as employed herein, "putative ligand" (also referred to as "test compound") refers to compounds such as steroid or steroid-like hormones, pharmaceutically active compounds, and the like, which are suspected to have the ability to bind to the receptor of
20 interest, and to modulate transcription of genes maintained under the control of response elements recognized by such receptor.

Examples of known ligands include all-trans-retinoic acid (ligand for retinoic acid receptor), 9-cis-
25 retinoic acid (ligand for retinoid X receptor), thyroid hormone (ligand for thyroid hormone receptor), 1,25-dihydroxy vitamin D₃ (ligand for vitamin D₃ receptor), and the like.

In accordance with another aspect of the present
30 invention, there is provided a method to repress the activity of a member of the steroid/thyroid hormone superfamily of receptors containing a silencing domain which represses basal level promoter activity of target genes, said method comprising contacting said member of the

steroid/thyroid hormone superfamily of receptors with a sufficient quantity of a co-suppressor as described hereinabove so as to repress the activity of said member. Members of the superfamily contemplated for repression in accordance with this aspect of the present invention include thyroid hormone receptor, retinoic acid receptor, vitamin D receptor, peroxisome proliferator activated receptor, and the like.

In accordance with yet another aspect of the present invention, there is provided a method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor as described hereinabove, said method comprising comparing the size of the above-described co-suppressor/dimeric receptor complex (i.e., complexes comprising the above-described co-suppressor and a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors) upon exposure to test compound, relative to the size of said complex in the absence of test compound. An observed size corresponding to intact complex is indicative of an inactive compound, while an observed size that reflects dissociation of the complex is indicative of a compound that disrupts the complex, thereby relieving the suppression caused thereby. Optionally, the complex employed in this assay further comprises a response element for said member of the steroid/thyroid hormone superfamily of receptors.

The size of the above-described complex can readily be determined employing various techniques available in the art. For example, electrophoretic mobility shift assays (EMSA) can be employed (wherein receptor alone or receptor-co-suppressor complex is bound to target DNA and the relative mobility thereof determined). Those of skill in the art can readily identify other methodology which can be employed to

determine the size of the complex as a result of exposure to putative ligand.

In accordance with a still further aspect of the present invention, there is provided a method to identify
5 compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor as described hereinabove, without substantially activating said receptor, said method comprising:

10 comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

15 a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or
20 retinoic acid receptor-retinoid X receptor heterodimer,

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is
25 operatively linked to a reporter, and

optionally, invention co-suppressor, and

wherein said second expression system comprises a complex comprising:

30 a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains

hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes,

5 the same response element-reporter combination as employed in said first expression system, and

optionally, invention co-suppressor, and thereafter

selecting those compounds which provide:

10 a higher reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of said compound, and

15 substantially the same reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of said compound,

wherein said selected compounds are capable of relieving the suppression of steroid/thyroid hormone
20 receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, but substantially lacking the ability to activate steroid/thyroid hormone receptor activity.

25 The addition of invention co-suppressor is optional in the above-described assay because it is present endogenously in most host cells employed for such assays. It is preferred, to ensure the presence of a fairly constant amount of co-suppressor, and to ensure that co-
30 suppressor is not a limiting reagent, that co-suppressor be supplied exogenously to the above-described assays.

Mutant receptors contemplated for use in the practice of the present invention are conveniently produced

by expression plasmids, introduced into the host cell by transfection. Mutant receptors contemplated for use herein include RAR403 homodimers, RAR403-containing heterodimers, TR160 homodimers, TR160-containing heterodimers, and the
5 like.

Reporter constructs contemplated for use in the practice of the present invention comprise:

- (a) a promoter that is operable in the host cell,
- 10 (b) a hormone response element, and
- (c) a DNA segment encoding a reporter protein,
wherein the reporter protein-encoding DNA segment is operatively linked to the
15 promoter for transcription of the DNA segment, and
wherein the hormone response element is operatively linked to the promoter for activation thereof.

20 Hormone response elements contemplated for use in the practice of the present invention are well known in the art, as has been noted previously.

Exemplary reporter genes include chloramphenicol transferase (CAT), luciferase (LUC), beta-galactosidase
25 (β -gal), and the like. Exemplary promoters include the simian virus (SV) promoter or modified form thereof (e.g., Δ SV), the thymidine kinase (TK) promoter, the mammary tumor virus (MTV) promoter or modified form thereof (e.g., Δ MTV), and the like [see, for example, Mangelsdorf et al., in
30 Nature 345:224-229 (1990), Mangelsdorf et al., in Cell 66:555-561 (1991), and Berger et al., in J. Steroid Biochem. Molec. Biol. 41:733-738 (1992)].

As used herein in the phrase "operative response element functionally linked to an operative reporter gene", the word "operative" means that the respective DNA sequences (represented by the terms "GAL4 response element" and "reporter gene") are operational, i.e., work for their intended purposes; the word "functionally" means that after the two segments are linked, upon appropriate activation by a ligand-receptor complex, the reporter gene will be expressed as the result of the fact that the "GAL4 response element" was "turned on" or otherwise activated.

In practicing the above-described functional bioassay, the expression plasmid and the reporter plasmid are co-transfected into suitable host cells. The transfected host cells are then cultured in the presence and absence of a test compound to determine if the test compound is able to produce activation of the promoter operatively linked to the response element of the reporter plasmid. Thereafter, the transfected and cultured host cells are monitored for induction (i.e., the presence) of the product of the reporter gene sequence.

Any cell line can be used as a suitable "host" for the functional bioassay contemplated for use in the practice of the present invention. Thus, cells contemplated for use in the practice of the present invention include transformed cells, non-transformed cells, neoplastic cells, primary cultures of different cell types, and the like. Exemplary cells which can be employed in the practice of the present invention include Schneider cells, CV-1 cells, HuTu80 cells, F9 cells, NTERA2 cells, NB4 cells, HL-60 cells, 293 cells, Hela cells, yeast cells, and the like. Preferred host cells for use in the functional bioassay system are COS cells and CV-1 cells. COS-1 (referred to as COS) cells are monkey kidney cells that express SV40 T antigen (Tag); while CV-1 cells do not express SV40 Tag. The presence of Tag in the COS-1

derivative lines allows the introduced expression plasmid to replicate and provides a relative increase in the amount of receptor produced during the assay period. CV-1 cells are presently preferred because they are particularly
5 convenient for gene transfer studies and provide a sensitive and well-described host cell system.

The above-described cells (or fractions thereof) are maintained under physiological conditions when contacted with physiologically active compound.
10 "Physiological conditions" are readily understood by those of skill in the art to comprise an isotonic, aqueous nutrient medium at a temperature of about 37°C.

In accordance with yet another aspect of the present invention, there is provided a method to identify
15 compounds which activate steroid/thyroid hormone receptor activity, but substantially lack the ability to relieve the suppression caused by a co-suppressor as described hereinabove, said method comprising:

comparing the reporter signal produced by two
20 different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a homodimeric or heterodimeric member
25 of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or
30 retinoic acid receptor-retinoid X receptor heterodimer,

a response element for said member of the steroid/thyroid hormone superfamily of

receptors, wherein said response element is operatively linked to a reporter, and optionally, invention co-suppressor, and

5 wherein said second expression system comprises a complex comprising:

a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein
10 said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes,

15 the same response element-reporter combination as employed in said first expression system, and

optionally, invention co-suppressor, and thereafter

20 selecting those compounds which provide:

a higher reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of compound, and

25 substantially the same reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of said compound,

wherein said selected compounds are capable of
30 activating steroid/thyroid hormone receptor activity, but substantially lacking the ability to relieve the suppression caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors.

In accordance with a still further aspect of the present invention, there is provided a method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor as
5 described hereinabove, and activate said receptor, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

10 wherein said first expression system comprises a complex comprising:

 a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone
15 receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

20 a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

 optionally, invention co-suppressor,
25 and

 wherein said second expression system comprises a complex comprising:

 a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed
30 in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level
35 promoter activity of target genes,

the same response element-reporter combination as employed in said first expression system, and

5 optionally, invention co-suppressor, and thereafter

selecting those compounds which provide:

10 increased reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of said compound, and

substantially increased reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of said compound,

15 wherein said selected compounds are capable of relieving the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, and
20 activating said receptor.

In accordance with still another embodiment of the present invention, there are provided modified forms of the above-described co-suppressor, including:

25 full length silencing mediator for retinoic acid and thyroid receptors plus GAL4 DNA binding domain,

full length silencing mediator for retinoic acid and thyroid receptors plus GAL4 activation domain,

30 full length silencing mediator for retinoic acid and thyroid receptors plus glutathione S-transferase (GST) tag, and the like.

The above-described modified forms of invention co-suppressor can be used in a variety of ways, e.g., in the assays described herein.

5 An especially preferred modified co-suppressor of the invention comprises full length silencing mediator for retinoic acid and thyroid receptors plus GAL4 activation domain.

10 In accordance with a still further embodiment of the present invention, there is provided a method to identify compounds which disrupt the ability of a co-suppressor as described hereinabove to complex with steroid/thyroid hormone receptors, without substantially activating said receptor, said method comprising:

15 comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

20 a modified co-suppressor as described above,

25 a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer or retinoic acid receptor-retinoid X receptor heterodimer, and

30 a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

wherein said second expression system comprises a complex comprising:

5 said modified co-suppressor,
 a homodimeric or heterodimeric form of
the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but
10 has lost its ability to repress basal level promoter activity of target genes, and
 the same response element-reporter combination as employed in said first expression system, and thereafter

15 selecting those compounds which provide:
 a lower reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of said compound, and
20 substantially the same reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of said compound,

25 wherein said selected compounds are capable of disrupting the ability of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors to complex with steroid/thyroid hormone receptors, without substantially activating said receptor.

30 Mutant receptors contemplated for use in this embodiment of the present invention include RAR403 homodimers, RAR403-containing heterodimers, TR160 homodimers, TR160-containing heterodimers, and the like.

Suitable host cells for use in this embodiment of the present invention include mammalian cells as well as yeast cells. Yeast cells are presently preferred because they introduce no background since SMRT (i.e., silencing mediator (co-repressor) for retinoic acid receptor (RAR) and thyroid hormone receptor (TR)) is not endogenous to yeast.

In accordance with yet another embodiment of the present invention, there is provided a method to identify compounds which activate steroid/thyroid hormone receptor activity, but substantially lack the ability to disrupt a complex comprising a steroid/thyroid hormone receptor and a co-suppressor as described hereinabove, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a modified co-suppressor as described above,

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer or retinoic acid receptor-retinoid X receptor heterodimer, and

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

wherein said second expression system comprises:

5 said modified co-suppressor,
 a homodimeric or heterodimeric form of
the same member of the steroid/thyroid
hormone superfamily of receptors as employed
in said first expression system, wherein
said member is mutated such that it retains
hormone dependent activation activity but
10 has lost its ability to repress basal level
promoter activity of target genes, and
 the same response element-reporter
combination as employed in said first
expression system, and thereafter

15 selecting those compounds which provide:
 a higher reporter signal upon exposure of
said compound to said second expression system,
relative to reporter signal in the absence of
compound, and
20 substantially the same reporter signal upon
exposure of said compound to said first
expression system, relative to reporter signal in
the absence of compound,

 wherein said selected compounds are capable of
25 activating steroid/thyroid hormone receptor activity, but
substantially lack the ability to disrupt the complex of a
co-suppressor having a structure and function
characteristic of the silencing mediator for retinoic acid
and thyroid receptors and a steroid/thyroid hormone
30 receptor.

Suitable host cells for use in this embodiment of
the present invention include mammalian cells as well as
yeast cells. Yeast cells are presently preferred because

they introduce no background since SMRT is not endogenous to yeast.

In accordance with a still further embodiment of the present invention, there is provided a method to
5 identify compounds which activate a steroid/thyroid hormone receptor, and disrupt the ability of a co-suppressor as described hereinabove to complex with said receptor, said method comprising:

10 comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

15 a modified co-suppressor as described above,

20 a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer or retinoic acid receptor-retinoid X receptor heterodimer, and

25 a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

wherein said second expression system comprises a complex comprising:

30 said modified co-suppressor,
the same homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member

is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes, and

5 the same response element-reporter combination as employed in said first expression system, and thereafter

selecting those compounds which provide:

10 a reduction in reporter signal upon exposure of compound to said first expression system, relative to reporter signal in the absence of said compound, and

15 increased reporter signal upon exposure of compound to said second expression system, relative to reporter signal in the absence of said compound,

20 wherein said selected compounds are capable of activating a steroid/thyroid hormone receptor and disrupting a complex comprising steroid/thyroid hormone receptor and a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors.

25 Suitable host cells for use in this embodiment of the present invention include mammalian cells as well as yeast cells. Yeast cells are presently preferred because they introduce no background since SMRT is not endogenous to yeast.

30 In accordance with yet another aspect of the present invention, there is provided a method to identify compounds which activate a steroid/thyroid hormone receptor and/or disrupt the ability of a co-suppressor as described hereinabove to complex with said receptor, said method comprising:

comparing the reporter signals produced by a combination expression system in the absence and presence of test compound,

5 wherein said combination expression system comprises:

10 a first homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

15 a second homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first homodimer or heterodimer, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes (i.e., provides basal level expression),

20 wherein either said first homodimer (or heterodimer) or said second homodimer (or heterodimer) is operatively linked to a GAL4 DNA binding domain,

25 a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a first reporter,

30 a GAL4 response element, wherein said response element is operatively linked to a second reporter, and

35

optionally a co-suppressor of steroid/thyroid hormone receptor activity, said co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, and thereafter

identifying as capable of relieving the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, but substantially lacking the ability to activate steroid/thyroid hormone receptor activity those compounds which provide:

a higher reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, and

substantially the same reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of said compound, or

identifying as capable of activating steroid/thyroid hormone receptor activity, but substantially lacking the ability to relieve the suppression caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors those compounds which provide:

a higher reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of compound, and

substantially the same reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, or

identifying as capable of relieving the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, and activating said receptor those compounds which provide:

a higher reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of said compound, and

a greater increase in reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound.

Thus, the change in expression level of the two different reporters introduced in a single transfection can be monitored simultaneously. Based on the results of this single transfection, one can readily identify the mode of interaction of test compound with the receptor/SMRT complex.

Exemplary GAL4 response elements are those containing the palindromic 17-mer:

5'-CGGAGGACTGTCCTCCG-3' (SEQ ID NO:3),

such as, for example, 17MX, as described by Webster et al., in Cell 52:169-178 (1988), as well as derivatives thereof.

Additional examples of suitable response elements include those described by Hollenberg and Evans in Cell 55:899-906 (1988); or Webster et al. in Cell 54:199-207 (1988).

In accordance with still another embodiment of the present invention, there is provided a method to identify compounds which activate a steroid/thyroid hormone receptor and/or disrupt the ability of a co-suppressor as described hereinabove to complex with said receptor, said method comprising:

10 comparing the reporter signals produced by a combination expression system in the absence and presence of test compound,

 wherein said combination expression system comprises:

15 a modified co-suppressor as described above,

 a first homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

25 a second homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first homodimer or heterodimer, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes,

30 wherein either said first
35 homodimer (or heterodimer) or said

second homodimer (or heterodimer) is operatively linked to a GAL4 DNA binding domain,

5 a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a first reporter,

10 a GAL4 response element, wherein said response element is operatively linked to a second reporter, and thereafter

identifying as capable of disrupting the ability of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors to complex with a steroid/thyroid hormone receptor, without substantially activating steroid/thyroid hormone receptor, those compounds which provide:

20 a lower reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, and

25 substantially the same reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of said compound, or

30 identifying as capable of activating steroid/thyroid hormone receptor activity, but substantially lacking the ability to disrupt a complex comprising a steroid/thyroid hormone receptor and a co-suppressor having a structure and function characteristic

of the silencing mediator for retinoic acid and thyroid receptors, those compounds which provide:

5 a higher reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of compound, and substantially the same reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, or

10 identifying as capable of disrupting a complex comprising a steroid/thyroid hormone receptor and a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, and activating said receptor those compounds which provide:

20 a reduction in reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, and

25 increased reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of said compound.

30 In accordance with a still further aspect of the present invention, there is provided a method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor as described hereinabove, said method comprising determining the effect of adding test compound to an expression system comprising:

a modified member of the steroid/thyroid hormone superfamily of receptors, wherein said modified member contains an activation domain which renders said receptor constitutively active,

5 a fusion protein comprising the receptor interaction domain of SMRT operatively linked to the GAL4 DNA binding domain, and

a GAL4 response element operatively linked to a reporter.

10 Prior to addition of an effective ligand for the member of the steroid/thyroid hormone superfamily of receptors employed herein, the association of the modified member and the fusion protein will be effective to bind the GAL4 response element and activate transcription of the
15 reporter. The presence of an effective ligand is indicated by a reduction of reporter signal upon exposure to ligand, which disrupts the interaction of the modified member and fusion protein.

Activation domains contemplated for use in the
20 practice of the present invention are well known in the art and can readily be identified by the artisan. Examples include the GAL4 activation domain, BP64, and the like.

To summarize, a novel nuclear receptor co-repressor which mediates the transcriptional silencing
25 of RAR and TR has been identified. This discovery is of great interest because transcriptional silencing has been shown to play an important role in development, cell differentiation and the oncogenic activity of v-erbA (Baniahmad et al., *EMBO J.* 11:1015-1023 (1992)); Gandrillon
30 et al., *Cell* 49:687-697 (1989)); Zenke et al., *Cell* 61:1035-1049 (1990); Barlow et al., *EMBO J.* 13:4241-4250 (1994); Levine and Manley, *Cell* 59:405-408 (1989); Baniahmad et al., *Proc. Natl. Acad. Sci. USA* 89:10633-10637 (1992b); and Saitou et al., *Nature* 374:159-162 (1995)). In

fact, v-erbA mutants that harbor the Pro160->Arg change in the TR neither suppress basal transcription nor are capable of oncogenic transformation (Damm and Evans, (1993) supra).

The function of SMRT as a silencing mediator
5 (co-repressor) of RAR and TR is analogous to mSin3 in the Mad-Max-Sin3 ternary complex (Schreiber-Agus et al., *Cell* 80:777-786 (1995); and Ayer et al., *Cell* 80:767-776 (1995)). Because GAL-SMRT functions as a potent repressor when bound to DNA, it is reasonable to speculate that the
10 function of the unliganded receptors is to bring with them SMRT to the template via protein-protein interaction. Thus, the repressor function is intrinsic to SMRT as opposed to the TR or RAR itself (Baniahmad et al., *Proc. Natl. Acad. Sci. USA* 90:8832-8836 (1993); and Fondell et
15 al., *Genes Dev* 7:1400-1410 (1993)). It is demonstrated herein that the ligand triggers a dissociation of SMRT from the receptor, which would lead to an initial step in the activation process. This would be followed (or be coincident) with an induced conformational change in the
20 carboxy-terminal transactivation domain (τ C, also called AF-2), allowing association with co-activators on the transcription machinery (Douarin et al., *EMBO J.* 14:2020-2033 (1995); Halachmi et al., *Science* 264:1455-1458 (1994); Lee et al., *Nature* 374:91-94 (1995); and Cavailles
25 et al., *Proc. Natl. Acad. Sci. USA* 91:10009-10013 (1994)). Thus, as has previously been suggested (Damm and Evans (1993) supra), the ligand dependent activation of TR would represent two separable processes including relief of repression and net activation. The isolation of SMRT now
30 provides a basis for dissecting the molecular basis of trans-repression.

The invention will now be described in greater detail by reference to the following non-limiting examples.

Example 1
Isolation of SMRT

Using a GAL4 DBD-RXR fusion protein (see, for example, USSN 08/177,740, incorporated by reference herein in its entirety) as a bait in a yeast two-hybrid screening system (Durfee et al. *Genes Dev* 7:555-569 (1993)), several cDNA clones encoding receptor interacting proteins were isolated. One of these proteins, SMRT, interacts strongly with unliganded RAR and TR but only weakly with RXR or other receptors in yeast. This protein was selected for further characterization.

Example 2
Far-western blotting procedure

Total bacteria extracts expressing GST fusions of hRAR α (aa 156-462) or hRXR α LBD (aa 228-462) and control extracts expressing GST alone or GST-PML fusion protein were subjected to SDS/PAGE and electroblotted onto nitrocellulose in transfer buffer (25 mM Tris, pH 8.3/ 192 mM glycine/ 0.01% SDS). After denaturation/renaturation from 6 M to 0.187 M guanidine hydrochloride in HB buffer (25 mM Hepes, pH 7.7/25 mM NaCl/5 mM MgCl₂/1mM DTT) filters were saturated at 4°C in blocking buffer (5% milk, then 1% milk in HB buffer plus 0.05% NP40). *In vitro* translated ³⁵S-labeled proteins were diluted into H buffer (20 mM Hepes, pH 7.7/75 mM KCl/0.1 mM EDTA/2.5 mM MgCl₂/0.05% NP40/ 1% milk/1 mM DTT) and the filters were hybridized overnight at 4°C with (1 μ M) or without ligand. After three washes with H buffer, filters were dried and exposed for autoradiography or quantitated by phosphoimager.

GST-SMRT is a GST fusion of the C-SMRT encoded by the yeast two hybrid clone. GST-SMRT has been purified, but contains several degradation products.

For yeast two-hybrid screening, a construct expressing the GAL4 DBD-hRXR α LBD (aa 198-462) fusion protein was used to screen a human lymphocyte cDNA library as described (Durfee et al., (1993) supra). Full length
5 SMRT cDNA was isolated from a human HeLa cDNA library (Clontech) using the two-hybrid insert as a probe.

Using the above-described far-western blotting procedure, 35 S-labeled SMRT preferentially complexes with
10 bacterial extracts expressing the RAR, marginally associates with RXR and shows no association with control extracts. In contrast, 35 S-PPAR selectively associates with its heterodimeric partner, RXR, but not with RAR. In a similar assay, 35 S-labeled RAR or TR interacts strongly with
15 SMRT and their heterodimeric partner, RXR, but not with degraded GST products, while 35 S-RXR interacts only weakly with SMRT. Binding of ligand to RAR or TR reduces their interactions with SMRT but not with RXR, while binding of ligand to RXR has only slight effect. Figure 1 shows the
20 quantitation of a dose-dependent dissociation of SMRT from RAR or TR by all-trans retinoic acid (atRA) or thyroid hormone (triiodothyronine or T3), demonstrating that the amount of ligand required for 50% dissociation in both cases are close to the kds for both ligands (Munoz et al.
25 *EMBO J.* 7:155-159 (1988); Sap et al., *Nature* 340:242-244 (1989); and Yang et al., *Proc. Natl. Acad. Sci. USA* 88:3559-3563 (1991)).

Full length SMRT encodes a polypeptide of 1495 amino acids rich in proline and serine residues (see Figure
30 2 and SEQ ID NO:1). Genbank database comparison reveals similarity of the C-terminal domain of SMRT to a partial cDNA encoding another receptor interacting protein, RIP13 (Seol et al., (1995) supra), whose role in receptor signaling is unknown. Within this region, there can be
35 identified several potential heptad repeats which might mediate protein-protein interaction with the "a-helical

sandwich" structure (Bourguet et al., *Nature* 375:377-382 (1995)) of the ligand binding domain (LBD) of receptors.

Example 3

Characterization of SMRT

5 Unlike other nuclear receptors, unliganded RAR and TR possess a strong silencing domain which represses basal level promoter activity of their target genes (Damm et al., *Nature* 339:593-597 (1989); Brent et al., *New Biol.* 1:329-336 (1989); Baniahmad et al., *Cell* 61:505-514 (1990);
10 and Baniahmad et al., *EMBO J.* 11:1015-1023 (1992)). The preferential interaction of SMRT with RAR and TR in the absence of hormone suggests that SMRT may play a role in mediating the transcriptional silencing effect of the receptor.

15 To further investigate the involvement of SMRT in silencing, the interaction of SMRT with mutant receptors which display distinct silencing and/or transactivation activities was tested as follows. ³⁵S-methionine labeled receptors were used as probes to hybridize immobilized
20 GST-SMRT in the presence (10 μ M) or absence of all-trans retinoic acid (atRA). The total bacteria extract expressing GST-RXR was included as a control.

 When quantitated by phosphoimager, RAR403 shows a 4-fold better interaction with SMRT than wild type RAR.
25 Both full length RAR or a deletion mutant expressing only the ligand binding domain (LBD, referred to as Δ AR) associate with SMRT; this association is blocked by ligand.

 These results confirm that the LBD alone is sufficient in the interaction. The carboxy-terminal
30 deletion mutant RAR403 is a potent dominant negative suppressor of basal level promoter activity of RAR target genes (Damm et al., *Proc. Natl. Acad. Sci. USA* 90:2989-2993

(1993); Tsai and Collins, *Proc. Natl. Acad. Sci. USA* 90:7153-7157 (1993); and Tsai et al., *Genes Dev* 6:2258-2269 (1992)). As might be predicted from the above studies, RAR403 and its amino terminal deletion derivative, $\Delta\Delta$ R403, interact strongly with SMRT in either the presence or absence of ligand, consistent with SMRT mediating the repressor activity of this mutant.

Example 4

Interaction of SMRT with TR Mutants

The interaction of SMRT with two different classes of TR mutants was analyzed next. The first mutant employed is the naturally occurring oncogene, v-erbA, which has strong silencing ability but no transactivation activity (Sap et al., (1989) *supra*; Sap et al., *Nature* 324:635-640 (1986); Weinberger et al., *Nature* 318:670-672 (1985); and Weinberger et al., *Nature* 324:641-646 (1986)). The second mutant employed is a single amino acid change (Pro 160 \rightarrow Arg) of the rTR α (TR160) which has previously been shown to lose its capacity in basal level suppression but retains hormone dependent transactivation (Thompson et al., *Science* 237:1610-1614 (1987); and Damm and Evans, *Proc. Natl. Acad. Sci. USA* 90:10668-10672 (1993)). If SMRT is involved in silencing, it would be expected that SMRT should interact with the v-erbA, but show little or no association with the silencing-defective TR160 mutant.

Interaction of the oncogenic v-erbA and rTR α R160 mutant (TR160) with GST-SMRT was determined in a far-western assay as described above (see Example 2). When quantitated by phosphoimager, the v-erbA shows an 18-fold better interaction with SMRT than hTR β , and the TR160 mutant shows a 10-fold lower signal than the rTR α .

As one might expect, v-erbA interacts strongly with SMRT both in presence or absence of ligand. In

contrast, full length TR160 mutant or LBD of TR160 ($\Delta\Delta$ TR160) does not interact significantly with SMRT when compared to the wild type receptor.

These data demonstrate that SMRT plays an important role in mediating transcriptional silencing effects of both RAR and TR. These data also suggest that the release of SMRT from receptors could be a prerequisite step in ligand-dependent transactivation by nuclear receptors.

10

Example 5

Formation of ternary complexes containing SMRT

RAR and TR form heterodimers with RXR, resulting in a complex with high DNA binding ability (Bugge et al., *EMBO J.* 11:1409-1418 (1992); Yu et al., *Cell* 67:1251-1266 (1991); and Kliewer et al., *Nature* 355:446-449 (1992)). Since SMRT interacts with RAR and TR, tests were conducted to determine whether SMRT can also interact with the receptor-DNA complex. Thus, the interaction of SMRT with RXR-RAR heterodimer on a DR5 element (i.e., an AGGTCA direct repeat spaced by five nucleotides) was determined in a gel retardation assay, which is carried out as follows. *In vitro* translated receptor or unprogrammed reticulocyte lysate (URL) was incubated with 1 μ g of poly dIdC on ice for 15 minutes in a total volume of 20 μ l containing 75 mM KCl, 7.5% glycerol, 20 mM Hepes (pH 7.5), 2 mM DTT and 0.1% NP-40, with or without ligand (in the range of about 10-100 nM employed). A 32 P labeled, double stranded oligonucleotide probe was added into the binding reaction (10,000 cpm per reaction), and the reaction was further incubated for 20 minutes at room temperature. The protein-DNA complex was separated on a 5% native polyacrylamide gel at 150 volts.

SMRT is seen to form a ternary complex with the RXR-RAR heterodimer on a DNA response element in the gel retardation assay. Addition of ligand releases SMRT from this complex in a dose-dependent manner.

- 5 Similarly, SMRT is seen to form a ternary complex with the RXR-TR heterodimer on a TR response element; addition of T3 disrupts the formation of this complex.

 These data demonstrate that SMRT can be recruited to DNA response elements via protein-protein interaction
10 with RAR or TR in the absence of hormone. Binding of hormone disrupts receptor-SMRT interaction and releases SMRT from the receptor-DNA complex.

Example 6

Transient transfection assay

- 15 CV-1 cells were plated in 24 well plates at a density of 50,000 cells per well. Expression plasmids were transfected into cells by lipofection using DOTAP. In each transfection, 5 ng of GAL-RAR and 15 ng of v-erbA or SMRT were used together with 150 ng of reporter construct
20 containing 4 copies of GAL4 binding sites in front of a minimal thymidine kinase promoter and a CMX- β -gal construct as an internal control. The relative luciferase activity was calculated by normalizing to the β -gal activity.

Example 7

Reversal of transcriptional silencing

 Recently, it has been shown that over expression of RAR or TR could reverse the transcriptional silencing effect of the GAL4 DBD fusion of TR (GAL-TR) or RAR (GAL-RAR) (Baniahmad et al., *Mol Cell Biol* 15:76-86 (1995); and Casanova et al., *Mol Cell Biol* 14:5756-5765 (1994)), presumably by competition for a limiting amount of a

co-repressor. A similar effect is observed herein when over expression of v-erbA or RAR403 mutants are shown to reverse the silencing effect of GAL-RAR and GAL-TR on the basal activity of a luciferase reporter (see Figure 3A and 3B).

In principle, over expression of SMRT should restore repressor activity when co-expressed with v-erbA or RAR403 competitors. Indeed, results presented in Figure 3C show that both the full length and the C-terminal domain of SMRT (C-SMRT) can titrate out v-erbA or RAR403 competitor activity and re-endow GAL-RAR and GAL-TR with silencing activity. In contrast, neither v-erbA nor SMRT show any effect on the transactivation activity of GAL-VP16 fusion. Thus, SMRT is able to block the titration effect of v-erbA and RAR403 and functionally replaces the putative co-repressor in this system.

Example 8

Direct recruitment of SMRT to a heterologous promoter

If SMRT is the mediator of transcription silencing of TR and RAR by interaction with template-bound unliganded receptors, then direct recruitment of SMRT to a heterologous promoter should result in repression of basal level activity. This was tested by fusing full length SMRT to the GAL4 DBD (GAL-SMRT). The effect of the resulting fusion protein on the activity of the thymidine kinase promoter containing four GAL4 binding sites was analyzed. Figure 3D shows that GAL-SMRT, like GAL-TR, can silence basal promoter activity in a dose-dependent manner. In contrast, GAL-RXR shows no suppression.

These data suggest that SMRT, when recruited to a promoter by direct DNA binding or via association with an unliganded receptor, functions as a potent transcriptional repressor.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Evans, Ronald M.
Chen, J. Don
- (ii) TITLE OF INVENTION: TRANSCRIPTIONAL CO-REPRESSOR THAT
INTERACTS WITH NUCLEAR HORMONE RECEPTORS AND USES THEREFOR
- (iii) NUMBER OF SEQUENCES: 3
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 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
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- (viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1495 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: both
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Ala	Gln	Lys	Leu	Pro	Gly	Asp	Pro	Pro	Cys	Trp	Thr	Ser	Gly	Leu	Pro
			20					25					30		
Phe	Pro	Val	Pro	Pro	Arg	Glu	Val	Ile	Lys	Ala	Ser	Pro	His	Ala	Pro
		35				40					45				
Asp	Pro	Ser	Ala	Phe	Ser	Tyr	Ala	Pro	Pro	Gly	His	Pro	Leu	Pro	Leu
		50				55					60				

41

Gly Leu His Asp Thr Ala Arg Pro Val Leu Pro Arg Pro Pro Thr Ile
 65 70 75 80
 Ser Asn Pro Pro Pro Leu Ile Ser Ser Ala Lys His Pro Ser Val Leu
 85 90 95
 Glu Arg Gln Ile Gly Ala Ile Ser Gln Gly Met Ser Val Gln Leu His
 100 105 110
 Val Pro Tyr Ser Glu His Ala Lys Ala Pro Val Gly Pro Val Thr Met
 115 120 125
 Gly Leu Pro Leu Pro Met Asp Pro Lys Lys Leu Ala Pro Phe Ser Gly
 130 135 140
 Val Lys Gln Glu Gln Leu Ser Pro Arg Gly Gln Ala Gly Pro Pro Glu
 145 150 155 160
 Ser Leu Gly Val Pro Thr Ala Gln Glu Ala Ser Val Leu Arg Gly Thr
 165 170 175
 Ala Leu Gly Ser Val Pro Gly Gly Ser Ile Thr Lys Gly Ile Pro Ser
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 Thr Arg Val Pro Ser Asp Ser Ala Ile Thr Tyr Arg Gly Ser Ile Thr
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 His Gly Thr Pro Ala Asp Val Leu Tyr Lys Gly Thr Ile Thr Arg Ile
 210 215 220
 Ile Gly Glu Asp Ser Pro Ser Arg Leu Asp Arg Gly Arg Glu Asp Ser
 225 230 235 240
 Leu Pro Lys Gly His Val Ile Tyr Glu Gly Lys Lys Gly His Val Leu
 245 250 255
 Ser Tyr Glu Gly Gly Met Ser Val Thr Gln Cys Ser Lys Glu Asp Gly
 260 265 270
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 275 280 285
 Tyr Asp Met Met Glu Gly Arg Val Gly Arg Ala Ile Ser Ser Ala Ser
 290 295 300
 Ile Glu Gly Leu Met Gly Arg Ala Ile Pro Pro Glu Arg His Ser Pro
 305 310 315 320
 His His Leu Lys Glu Gln His His Ile Arg Gly Ser Ile Thr Gln Gly
 325 330 335
 Ile Pro Arg Ser Tyr Val Glu Ala Gln Glu Asp Tyr Leu Arg Arg Glu
 340 345 350
 Ala Lys Leu Leu Lys Arg Glu Gly Thr Pro Pro Pro Pro Pro Pro Ser
 355 360 365
 Arg Asp Leu Thr Glu Ala Tyr Lys Thr Gln Ala Leu Gly Pro Leu Lys
 370 375 380
 Leu Lys Pro Ala His Glu Gly Leu Val Ala Thr Val Lys Glu Ala Gly
 385 390 395 400
 Arg Ser Ile His Glu Ile Pro Arg Glu Glu Leu Arg His Thr Pro Glu
 405 410 415

Leu Pro Leu Ala Pro Arg Pro Leu Lys Glu Gly Ser Ile Thr Gln Gly
 420 425 430
 Thr Pro Leu Lys Tyr Asp Thr Gly Ala Ser Thr Thr Gly Ser Lys Lys
 435 440 445
 His Asp Val Arg Ser Leu Ile Gly Ser Pro Gly Arg Thr Phe Pro Pro
 450 455 460
 Val His Pro Leu Asp Val Met Ala Asp Ala Arg Ala Leu Glu Arg Ala
 465 470 475 480
 Cys Tyr Glu Glu Ser Leu Lys Ser Arg Pro Gly Thr Ala Ser Ser Ser
 485 490 495
 Gly Gly Ser Ile Ala Arg Gly Ala Pro Val Ile Val Pro Glu Leu Gly
 500 505 510
 Lys Pro Arg Gln Ser Pro Leu Thr Tyr Glu Asp His Gly Ala Pro Phe
 515 520 525
 Ala Gly His Leu Pro Arg Gly Ser Pro Val Thr Met Arg Glu Pro Thr
 530 535 540
 Pro Arg Leu Gln Glu Gly Ser Leu Ser Ser Ser Lys Ala Ser Gln Asp
 545 550 555 560
 Arg Lys Leu Thr Ser Thr Pro Arg Glu Ile Ala Lys Ser Pro His Ser
 565 570 575
 Thr Val Pro Glu His His Pro His Pro Ile Ser Pro Tyr Glu His Leu
 580 585 590
 Leu Arg Gly Val Ser Gly Val Asp Leu Tyr Arg Ser His Ile Pro Leu
 595 600 605
 Ala Phe Asp Pro Thr Ser Ile Pro Arg Gly Ile Pro Leu Asp Ala Ala
 610 615 620
 Ala Ala Tyr Tyr Leu Pro Arg His Leu Ala Pro Asn Pro Thr Tyr Pro
 625 630 635 640
 His Leu Tyr Pro Pro Tyr Leu Ile Arg Gly Tyr Pro Asp Thr Ala Ala
 645 650 655
 Leu Glu Asn Arg Gln Thr Ile Ile Asn Asp Tyr Ile Thr Ser Gln Gln
 660 665 670
 Met His His Asn Thr Ala Thr Ala Met Ala Gln Arg Ala Asp Met Leu
 675 680 685
 Arg Gly Leu Ser Pro Arg Glu Ser Ser Leu Ala Leu Asn Tyr Ala Ala
 690 695 700
 Gly Pro Arg Gly Ile Ile Asp Leu Ser Gln Val Pro His Leu Pro Val
 705 710 715 720
 Leu Val Pro Pro Thr Pro Gly Thr Pro Ala Thr Ala Met Asp Arg Leu
 725 730 735
 Ala Tyr Leu Pro Thr Ala Pro Gln Pro Phe Ser Ser Arg His Ser Ser
 740 745 750
 Ser Pro Leu Ser Pro Gly Gly Pro Thr His Leu Thr Lys Pro Thr Thr
 755 760 765

Thr Ser Ser Ser Glu Arg Glu Arg Asp Arg Asp Arg Glu Arg Asp Arg
 770 775 780
 Asp Arg Glu Arg Glu Lys Ser Ile Leu Thr Ser Thr Thr Thr Val Glu
 785 790 795 800
 His Ala Pro Ile Trp Arg Pro Gly Thr Glu Gln Ser Ser Gly Ser Ser
 805 810 815
 Gly Ser Ser Gly Gly Gly Gly Gly Ser Ser Ser Arg Pro Ala Ser His
 820 825 830
 Ser His Ala His Gln His Ser Pro Ile Ser Pro Arg Thr Gln Asp Ala
 835 840 845
 Leu Gln Gln Arg Pro Ser Val Leu His Asn Thr Gly Met Lys Gly Ile
 850 855 860
 Ile Thr Ala Val Glu Pro Ser Lys Pro Thr Val Leu Arg Ser Thr Ser
 865 870 875 880
 Thr Ser Ser Pro Val Arg Pro Ala Ala Thr Phe Pro Pro Ala Thr His
 885 890 895
 Cys Pro Leu Gly Gly Thr Leu Asp Gly Val Tyr Pro Thr Leu Met Glu
 900 905 910
 Pro Val Leu Leu Pro Lys Glu Ala Pro Arg Val Ala Arg Pro Glu Arg
 915 920 925
 Pro Arg Ala Asp Thr Gly His Ala Phe Leu Ala Lys Pro Pro Ala Arg
 930 935 940
 Ser Gly Leu Glu Pro Ala Ser Ser Pro Ser Lys Gly Ser Glu Pro Arg
 945 950 955 960
 Pro Leu Val Pro Pro Val Ser Gly His Ala Thr Ile Ala Arg Thr Pro
 965 970 975
 Ala Lys Asn Leu Ala Pro His His Ala Ser Pro Asp Pro Pro Ala Pro
 980 985 990
 Pro Ala Ser Ala Ser Asp Pro His Arg Glu Lys Thr Gln Ser Lys Pro
 995 1000 1005
 Phe Ser Ile Gln Glu Leu Glu Leu Arg Ser Leu Gly Tyr His Gly Ser
 1010 1015 1020
 Ser Tyr Ser Pro Glu Gly Val Glu Pro Val Ser Pro Val Ser Ser Pro
 1025 1030 1035 1040
 Ser Leu Thr His Asp Lys Gly Leu Pro Lys His Leu Glu Glu Leu Asp
 1045 1050 1055
 Lys Ser His Leu Glu Gly Glu Leu Arg Pro Lys Gln Pro Gly Pro Val
 1060 1065 1070
 Lys Leu Gly Gly Glu Ala Ala His Leu Pro His Leu Arg Pro Leu Pro
 1075 1080 1085
 Glu Ser Gln Pro Ser Ser Ser Pro Leu Leu Gln Thr Ala Pro Gly Val
 1090 1095 1100
 Lys Gly His Gln Arg Val Val Thr Leu Ala Gln His Ile Ser Glu Val
 1105 1110 1115 1120

Ile Thr Gln Asp Tyr Thr Arg His His Pro Gln Gln Leu Ser Ala Pro
 1125 1130 1135
 Leu Pro Ala Pro Leu Tyr Ser Phe Pro Gly Ala Ser Cys Pro Val Leu
 1140 1145 1150
 Asp Leu Arg Arg Pro Pro Ser Asp Leu Tyr Leu Pro Pro Pro Asp His
 1155 1160 1165
 Gly Ala Pro Ala Arg Gly Ser Pro His Ser Glu Gly Gly Lys Arg Ser
 1170 1175 1180
 Pro Glu Pro Asn Lys Thr Ser Val Leu Gly Gly Gly Glu Asp Gly Ile
 1185 1190 1195 1200
 Glu Pro Val Ser Pro Pro Glu Gly Met Thr Glu Pro Gly His Ser Arg
 1205 1210 1215
 Ser Ala Val Tyr Pro Leu Leu Tyr Arg Asp Gly Glu Gln Thr Glu Pro
 1220 1225 1230
 Ser Arg Met Gly Ser Lys Ser Pro Gly Asn Thr Ser Gln Pro Pro Ala
 1235 1240 1245
 Phe Phe Ser Lys Leu Thr Glu Ser Asn Ser Ala Met Val Lys Ser Lys
 1250 1255 1260
 Lys Gln Glu Ile Asn Lys Lys Leu Asn Thr His Asn Arg Asn Glu Pro
 1265 1270 1275 1280
 Glu Tyr Asn Ile Ser Gln Pro Gly Thr Glu Ile Phe Asn Met Pro Ala
 1285 1290 1295
 Ile Thr Gly Thr Gly Leu Met Thr Tyr Arg Ser Gln Ala Val Gln Glu
 1300 1305 1310
 His Ala Ser Thr Asn Met Gly Leu Glu Ala Ile Ile Arg Lys Ala Leu
 1315 1320 1325
 Met Gly Lys Tyr Asp Gln Trp Glu Glu Ser Pro Pro Leu Ser Ala Asn
 1330 1335 1340
 Ala Phe Asn Pro Leu Asn Ala Ser Ala Ser Leu Pro Ala Ala Met Pro
 1345 1350 1355 1360
 Ile Thr Ala Ala Asp Gly Arg Ser Asp His Thr Leu Thr Ser Pro Gly
 1365 1370 1375
 Gly Gly Gly Lys Ala Lys Val Ser Gly Arg Pro Ser Ser Arg Lys Ala
 1380 1385 1390
 Lys Ser Pro Ala Pro Gly Leu Ala Ser Gly Asp Arg Pro Pro Ser Val
 1395 1400 1405
 Ser Ser Val His Ser Glu Gly Asp Cys Asn Arg Arg Thr Pro Leu Thr
 1410 1415 1420
 Asn Arg Val Trp Glu Asp Arg Pro Ser Ser Ala Gly Ser Thr Pro Phe
 1425 1430 1435 1440
 Pro Tyr Asn Pro Leu Ile Met Arg Leu Gln Ala Gly Tyr Met Ala Ser
 1445 1450 1455
 Pro Pro Pro Pro Gly Leu Pro Ala Gly Ser Gly Pro Leu Ala Gly Pro

45

1460

1465

1470

His His Ala Trp Asp Glu Glu Pro Lys Pro Leu Leu Cys Ser Gln Tyr
1475 1480 1485

Glu Thr Leu Ser Asp Ser Glu
1490 1495

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gly Lys Tyr Asp Gln Trp Glu Glu Ser Pro Pro Leu Ser Ala Asn Ala
1 5 10 15

Phe Asn Pro Leu Asn Ala Ser Ala Ser Leu Pro Ala Ala Met Pro Ile
20 25 30

Thr Ala Ala Asp Gly Arg Ser Asp His Thr Leu Thr Ser Pro
35 40 45

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGGAGGACTG TCCTCCG

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That which is claimed is:

1. A co-suppressor of steroid/thyroid hormone receptor activity, said co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors.
2. A co-suppressor according to claim 1 having substantially the same sequence as residues 1-1329 plus 1376-1495, as set forth in SEQ ID NO:1.
3. A co-suppressor according to claim 2 further comprising the amino acid residues set forth in SEQ ID NO:2, i. e., residues 1330-1375 of SEQ ID NO:1.
4. An antibody raised against the co-suppressor of claim 1.
5. A method to block the repressing effect of co-suppressors having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, said method comprising administering an effective amount of an antibody according to claim 4.
6. Isolated polynucleic acid encoding the co-suppressor of claim 1.
7. A vector containing polynucleic acid according to claim 6.
8. A complex comprising the co-suppressor of claim 1 and a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors, wherein said member contains a silencing domain which represses basal level promoter activity of target genes.

9. A complex according to claim 8 wherein said homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors is selected from thyroid hormone receptor homodimer, thyroid hormone receptor-
5 retinoid X receptor heterodimer, retinoic acid receptor homodimer, retinoic acid receptor-retinoid X receptor heterodimer or retinoid X receptor homodimer.

10. A complex according to claim 8 further comprising a response element for said member of the steroid/thyroid hormone superfamily of receptors.

11. A method to dissociate the complex of claim 8, said method comprising contacting said complex with ligand for said member of the steroid/thyroid hormone superfamily of receptors.

12. A method to repress the activity of a member of the steroid/thyroid hormone superfamily of receptors containing a silencing domain which represses basal level promoter activity of target genes, said method comprising
5 contacting said member of the steroid/thyroid hormone superfamily of receptors with a sufficient quantity of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors so as to repress the activity of said
10 member.

13. A method according to claim 12 wherein said member of the steroid/thyroid hormone superfamily of receptors is selected from thyroid hormone receptor, retinoic acid receptor, vitamin D receptor or peroxisome
5 proliferator activated receptor.

14. A method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, said method comprising
5 comparing the size of the complex of claim 8 upon exposure to test compound, relative to the size of said complex in the absence of test compound.

15. A method according to claim 14 wherein said complex further comprises a response element for said member of the steroid/thyroid hormone superfamily of receptors.

16. A method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for
5 retinoic acid and thyroid receptors, without substantially activating said receptor, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

10 wherein said first expression system comprises a complex comprising:

a homodimeric or heterodimeric member
of the steroid/thyroid hormone superfamily
of receptors selected from thyroid hormone
15 receptor homodimer, thyroid hormone
receptor-retinoid X receptor heterodimer,
retinoic acid receptor homodimer, or
retinoic acid receptor-retinoid X receptor
heterodimer,

20 a response element for said member of
the steroid/thyroid hormone superfamily of

receptors, wherein said response element is
operatively linked to a reporter, and

25 optionally, the co-suppressor of claim
1, and

wherein said second expression system
comprises a complex comprising:

30 a homodimeric or heterodimeric form of
the same member of the steroid/thyroid
hormone superfamily of receptors as employed
in said first expression system, wherein
said member is mutated such that it retains
hormone dependent activation activity but
has lost its ability to repress basal level
35 promoter activity of target genes,

the same response element-reporter
combination as employed in said first
expression system, and

40 optionally, the co-suppressor of claim
1, and thereafter

selecting those compounds which provide:

45 a higher reporter signal upon exposure of
said compound to said first expression system,
relative to reporter signal in the absence of
said compound, and

substantially the same reporter signal upon
exposure of said compound to said second
expression system, relative to reporter signal in
the absence of said compound,

50 wherein said selected compounds are capable of
relieving the suppression of steroid/thyroid hormone
receptor activity caused by a co-suppressor having a
structure and function characteristic of the silencing
mediator for retinoic acid and thyroid receptors, but

55 substantially lacking the ability to activate steroid/thyroid hormone receptor activity.

17. A method according to claim 16 wherein said mutant receptor is selected from RAR403 homodimers, RAR403-containing heterodimers, TR160 homodimers or TR160-containing heterodimers.

18. A method to identify compounds which activate steroid/thyroid hormone receptor activity, but substantially lack the ability to relieve the suppression caused by a co-suppressor having a structure and function
5 characteristic of the silencing mediator for retinoic acid and thyroid receptors, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

10 wherein said first expression system comprises a complex comprising:

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone
15 receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

20 a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

optionally, the co-suppressor of claim
25 1, and

wherein said second expression system comprises a complex comprising:

51

30 a homodimeric or heterodimeric form of
the same member of the steroid/thyroid
hormone superfamily of receptors as employed
in said first expression system, wherein
said member is mutated such that it retains
hormone dependent activation activity but
has lost its ability to repress basal level
35 promoter activity of target genes,
the same response element-reporter
combination as employed in said first
expression system, and
optionally, the co-suppressor of claim
40 1, and thereafter

selecting those compounds which provide:

45 a higher reporter signal upon exposure of
said compound to said second expression system,
relative to reporter signal in the absence of
compound, and

substantially the same reporter signal upon
exposure of said compound to said first
expression system, relative to reporter signal in
the absence of said compound,

50 wherein said selected compounds are capable of
activating steroid/thyroid hormone receptor activity, but
substantially lacking the ability to relieve the
suppression caused by a co-suppressor having a structure
and function characteristic of the silencing mediator for
55 retinoic acid and thyroid receptors.

19. A method to identify compounds which relieve
the suppression of steroid/thyroid hormone receptor
activity caused by a co-suppressor having a structure and
function characteristic of the silencing mediator for
5 retinoic acid and thyroid receptors, and activate said
receptor, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

10 wherein said first expression system comprises a complex comprising:

 a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone
15 receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

20 a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

25 optionally, the co-suppressor of claim 1, and

 wherein said second expression system comprises a complex comprising:

 a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed
30 in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes,
35 the same response element-reporter combination as employed in said first expression system, and

 optionally, the co-suppressor of claim
40 1, and thereafter

selecting those compounds which provide:

increased reporter signal upon exposure of
said compound to said second expression system,
relative to reporter signal in the absence of
said compound, and

45

substantially increased reporter signal upon
exposure of said compound to said first
expression system, relative to reporter signal in
the absence of said compound,

50

wherein said selected compounds are capable of
relieving the suppression of steroid/thyroid hormone
receptor activity caused by a co-suppressor having a
structure and function characteristic of the silencing
mediator for retinoic acid and thyroid receptors, and
activating said receptor.

55

20. A modified form of the co-suppressor of
claim 1 selected from:

5

full length silencing mediator for retinoic acid
and thyroid receptors plus GAL4 DNA binding
domain,

full length silencing mediator for retinoic acid
and thyroid receptors plus GAL4 activation
domain, or

10

full length silencing mediator for retinoic acid
and thyroid receptors plus glutathione
S-transferase (GST) tag.

21. A modified co-suppressor according to claim
20, wherein said modified co-suppressor comprises full
length silencing mediator for retinoic acid and thyroid
receptors plus GAL4 activation domain.

22. A method to identify compounds which disrupt the ability of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors to complex with
5 steroid/thyroid hormone receptors, without substantially activating said receptor, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

10 wherein said first expression system comprises a complex comprising:

a modified co-suppressor according to claim 21,

15 a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer or retinoic
20 acid receptor-retinoid X receptor heterodimer, and

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is
25 operatively linked to a reporter, and

wherein said second expression system comprises a complex comprising:

said modified co-suppressor,

30 a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but

35 has lost its ability to repress basal level
promoter activity of target genes, and
 the same response element-reporter
combination as employed in said first
expression system, and thereafter

40 selecting those compounds which provide:
 a lower reporter signal upon exposure of
said compound to said first expression system,
relative to reporter signal in the absence of
said compound, and

45 substantially the same reporter signal upon
exposure of said compound to said second
expression system, relative to reporter signal in
the absence of said compound,

 wherein said selected compounds are capable of
50 disrupting the ability of a co-suppressor having a
structure and function characteristic of the silencing
mediator for retinoic acid and thyroid receptors to complex
with steroid/thyroid hormone receptors, without
substantially activating said receptor.

23. A method according to claim 22 wherein said
mutant receptor is selected from RAR403 homodimers, RAR403-
containing heterodimers, TR160 homodimers or TR160-
containing heterodimers.

24. A method according to claim 22, wherein the
host is a mammalian or yeast cell.

25. A method to identify compounds which activate steroid/thyroid hormone receptor activity, but substantially lack the ability to disrupt a complex comprising a steroid/thyroid hormone receptor and a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a modified co-suppressor according to claim 21,

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer or retinoic acid receptor-retinoid X receptor heterodimer, and

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

wherein said second expression system comprises:

said modified co-suppressor, a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but

has lost its ability to repress basal level promoter activity of target genes, and
the same response element-reporter combination as employed in said first expression system, and thereafter

40

selecting those compounds which provide:

45

a higher reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of compound, and

substantially the same reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of compound,

50

wherein said selected compounds are capable of activating steroid/thyroid hormone receptor activity, but substantially lack the ability to disrupt the complex of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors and a steroid/thyroid hormone receptor.

55

26. A method according to claim 25, wherein the host is a mammalian or yeast cell.

27. A method to identify compounds which activate a steroid/thyroid hormone receptor, and disrupt the ability of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors to complex with said receptor, said method comprising:

5

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

10 wherein said first expression system
comprises a complex comprising:

 a modified co-suppressor according to
claim 21,

15 a homodimeric or heterodimeric member
of the steroid/thyroid hormone superfamily
of receptors selected from thyroid hormone
receptor homodimer, thyroid hormone
receptor-retinoid X receptor heterodimer,
20 retinoic acid receptor homodimer or retinoic
acid receptor-retinoid X receptor
heterodimer, and

 a response element for said member of
the steroid/thyroid hormone superfamily of
receptors, wherein said response element is
25 operatively linked to a reporter, and

 wherein said second expression system
comprises a complex comprising:

 said modified co-suppressor,

30 the same homodimeric or heterodimeric
member of the steroid/thyroid hormone
superfamily of receptors as employed in said
first expression system, wherein said member
is mutated such that it retains hormone
dependent activation activity but has lost
35 its ability to repress basal level promoter
activity of target genes, and

 the same response element-reporter
combination as employed in said first
expression system, and thereafter

40 selecting those compounds which provide:

 a reduction in reporter signal upon exposure
of compound to said first expression system,
relative to reporter signal in the absence of
said compound, and

45 increased reporter signal upon exposure of
compound to said second expression system,
relative to reporter signal in the absence of
said compound,

 wherein said selected compounds are capable of
50 activating a steroid/thyroid hormone receptor and
disrupting a complex comprising steroid/thyroid hormone
receptor and a co-suppressor having a structure and
function characteristic of the silencing mediator for
retinoic acid and thyroid receptors.

28. A method according to claim 27, wherein the
host is a mammalian or yeast cell.

29. A method to identify compounds which
activate a steroid/thyroid hormone receptor and/or disrupt
the ability of a co-suppressor having a structure and
function characteristic of the silencing mediator for
5 retinoic acid and thyroid receptors to complex with said
receptor, said method comprising:

 comparing the reporter signals produced by a
combination expression system in the absence and presence
of test compound,

10 wherein said combination expression system
comprises:

 a first homodimeric or heterodimeric
member of the steroid/thyroid hormone
superfamily of receptors selected from
15 thyroid hormone receptor homodimer, thyroid
hormone receptor-retinoid X receptor
heterodimer, retinoic acid receptor
homodimer, or retinoic acid receptor-
retinoid X receptor heterodimer,

20 a second homodimeric or heterodimeric
form of the same member of the

steroid/thyroid hormone superfamily of
receptors as employed in said first
homodimer or heterodimer, wherein said
25 member is mutated such that it retains
hormone dependent activation activity but
has lost its ability to repress basal level
promoter activity of target genes,

wherein either said first
30 homodimer (or heterodimer) or said
second homodimer (or heterodimer) is
operatively linked to a GAL4 DNA
binding domain,

a response element for said member of
35 the steroid/thyroid hormone superfamily of
receptors, wherein said response element is
operatively linked to a first reporter,

a GAL4 response element, wherein said
response element is operatively linked to a
40 second reporter, and thereafter

identifying as capable of relieving the
suppression of steroid/thyroid hormone receptor activity
caused by a co-suppressor having a structure and function
characteristic of the silencing mediator for retinoic acid
45 and thyroid receptors, but substantially lacking the
ability to activate steroid/thyroid hormone receptor
activity those compounds which provide:

a higher reporter signal from the reporter
responsive to the first member upon exposure of
50 said compound to said first member, relative to
reporter signal in the absence of said compound,
and

substantially the same reporter signal from
the reporter responsive to the second member upon
55 exposure of said compound to said second member,

relative to reporter signal in the absence of said compound, or

identifying as capable of activating steroid/thyroid hormone receptor activity, but
60 substantially lacking the ability to relieve the suppression caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors those compounds which provide:

65 a higher reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of compound, and
70 substantially the same reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, or

identifying as capable of relieving the
75 suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, and activating said receptor those compounds which provide:

80 increased reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of said compound, and

85 substantially increased reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound.

30. A method according to claim 29 wherein said combination expression system further comprises a co-suppressor of steroid/thyroid hormone receptor activity, said co-suppressor having a structure and function
5 characteristic of the silencing mediator for retinoic acid and thyroid receptors.

31. A method to identify compounds which activate a steroid/thyroid hormone receptor and/or disrupt the ability of a co-suppressor having a structure and function characteristic of the silencing mediator for
5 retinoic acid and thyroid receptors to complex with said receptor, said method comprising:

comparing the reporter signals produced by a combination expression system in the absence and presence of test compound,

10 wherein said combination expression system comprises:

a modified co-suppressor according to claim 21,

15 a first homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor
20 homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

25 a second homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first homodimer or heterodimer, wherein said member is mutated such that it retains hormone dependent activation activity but

30 has lost its ability to repress basal level
promoter activity of target genes,

wherein either said first
homodimer (or heterodimer) or said
second homodimer (or heterodimer) is
operatively linked to a GAL4 DNA
35 binding domain,

a response element for said member of
the steroid/thyroid hormone superfamily of
receptors, wherein said response element is
operatively linked to a first reporter,

40 a GAL4 response element, wherein said
response element is operatively linked to a
second reporter, and thereafter

identifying as capable of disrupting the ability
of a co-suppressor having a structure and function
45 characteristic of the silencing mediator for retinoic acid
and thyroid receptors to complex with steroid/thyroid
hormone receptor, without substantially activating
steroid/thyroid hormone receptor, those compounds which
provide:

50 a lower reporter signal from the reporter
responsive to the first member upon exposure of
said compound to said first member, relative to
reporter signal in the absence of said compound,
and

55 substantially the same reporter signal from
the reporter responsive to the second member upon
exposure of said compound to said second member,
relative to reporter signal in the absence of
said compound, or

60 identifying as capable of activating
steroid/thyroid hormone receptor activity, but

substantially lacking the ability to disrupt a complex comprising a steroid/thyroid hormone receptor and a co-suppressor having a structure and function characteristic
65 of the silencing mediator for retinoic acid and thyroid receptors those compounds which provide:

a higher reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to
70 reporter signal in the absence of compound, and
substantially the same reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of
75 said compound, or

identifying as capable of disrupting a complex comprising a steroid/thyroid hormone receptor and a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid
80 receptors, and activating said receptor those compounds which provide:

a reduction in reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of
85 said compound, and

increased reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to
90 reporter signal in the absence of said compound.

32. A method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, said method comprising
5 determining the effect of adding test compound to an expression system comprising:

a modified member of the steroid/thyroid hormone superfamily of receptors, wherein said modified member
10 contains an activation domain which renders said receptor constitutively active,

a fusion protein comprising the receptor interaction domain of SMRT operatively linked to the GAL4 DNA binding domain, and

15 a GAL4 response element operatively linked to a reporter.

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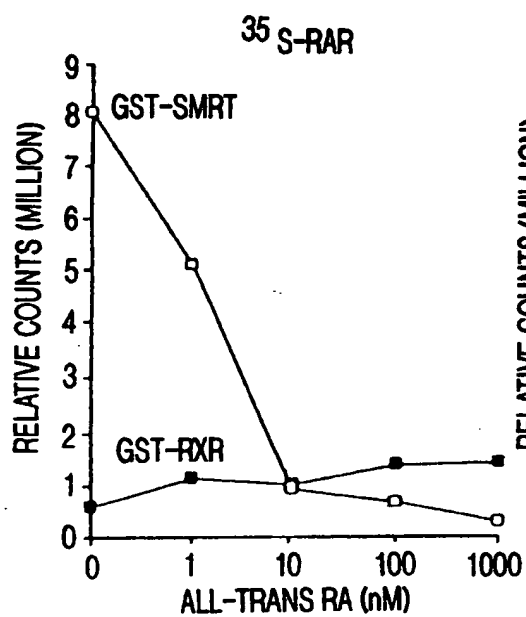


FIG. 1A

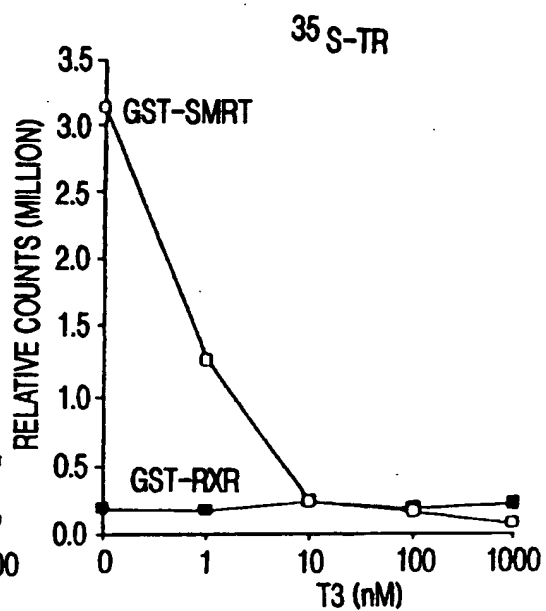


FIG. 1B

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1 HEAWDAH@DKEAFAAEAQKLE@G@B@C@WTSGL@F@V@P@P@REVIKAS@H@A@B@
 51 SAFSYA@B@G@H@B@L@LGLHDTAR@V@L@P@B@T@ISN@B@P@L@ISSAKH@B@SVLERQI
 101 GAISQGMVSQV@LHV@YSEHAKA@V@Q@V@TMGL@B@L@P@M@P@K@K@L@B@F@SGVKQEQ@L
 151 SPRGQAGPPESLGVPTAQEASVLRGTALG@SVPGGSITKGI@PSTRVPSDSA
 201 ITYRGSITHGTPADVLYKGTITRIIGEDSPSRLDRGRED@SLPKGHVIYEG
 251 KKGHVLSYEGGMSV@TQCSKEDGRSSSGPPHETAAPKRTYD@MEGRVGRAI
 301 SSASIEGLMGRAIPPERHSPHHLKEQH@H@IRGSITQGI@PRSYVEAQEDYLR
 351 REAKLLKREGTPPPPPPSRDLTEAYKTQALG@PLK@L@K@PAHEGLVATVKEAG
 401 RSIHEIPREELRHTPELPLAPRPLKEGSITQGTPLKYDTGASTTGSKKHD
 451 VRS@LIGSPGRTFPPVHPLDVMADARALERACYEESL@KSRPGTASSSGGSI
 501 ARGAPVIVPELGKPRQSPLTYEDHGAPFAGHLPRGSPV@TMREPTPRLQEG
 551 SLSSSKASQDRKLTSTPREIAKSPHSTVPEH@H@PH@P@ISPYEHL@RGVSGVD
 601 LYRSHIPLAFDPTSI@PRGIPLDAAAAY@LPRHLAPNPTYPHLYPPY@LIRG
 651 YPD@TAAL@NRQTIINDYITSQQMHHNTATAMAQRADMLRGLSPRESSLAL
 701 NYAAGPRGIIDLSQVPHLPVLVPPTPGTPATAM@DRLAYLPTAPQPFSSRH
 751 SSSPLSPCGP@THLTKPTTSSSERERDRDRERDREREK@SILTSTTTVE
 801 HAPIWRPGTEQSSGSSGSSGGGGSSSRPASHSHAHQHSPI@SPRTQDALQ
 851 QRPSVLHNTGMKGIITAVEPSKPTVLRSTSTSSPVRPAATFPPATHCPLG
 901 GTLDGVYPTLHEPVLLPKEAPRVARPERPRADTGHAF@LAKPPARSGLEPA
 951 SSPSKGSEPRPLVPPVSGHAT@IARTPAK@NLAPHHASPDPPAPPASASDPH
 1001 REKTQSKPFSIQELELRSLGYHGSSYSPEGVEPVSPVSSPSLTHDKGLPK
 1051 HLEELDKSHLEGELRPKQPGPVKLGGEAAHLPHLRPLPES@PSSSPLI@P
 1101 APGVKGH@RVVTLA@HISEVIT@DYTRHHE@L@SAPLPAPLYSFP@GASCP
 1151 VLDLRRPPSDLYLPPPDHGAPARGSPHSEGGKRSPEPNKTSVLGGGEDGI
 1201 EPVSPPEGMT@PGHSRS@VYPLLYRDGEQTEPSRMGSKSPGNTSQPPAFF

 1251 SK@TESNSA@V@KSKKQ@B@NKKLNT@N@NRNEPEYNISQPGTEIFNMPAITGT
 1301 GL@TYR@Q@V@DEHASTNMGLEA@IRKALM@CKYD@W@EESP@LSANAFNPL
 1350 NASASLPAAMPITAADGRSDHTLTSP|GGGGKAKVSGRPSSRKAKSPAPG
 1399 LA...SGDRPPSVSSVHSEGD@CNRRTP@LTNRVWEDRPSSAGSTPF@PYNPL@I
 1447 MRLQAG@V@MASPPPP@P@PAGSGP@...AGPHHA...WDEEPKPL@CSQYET@

1492 SDSE* 1495

FIG. 2
 SUBSTITUTE SHEET (RULE 26)

3/3

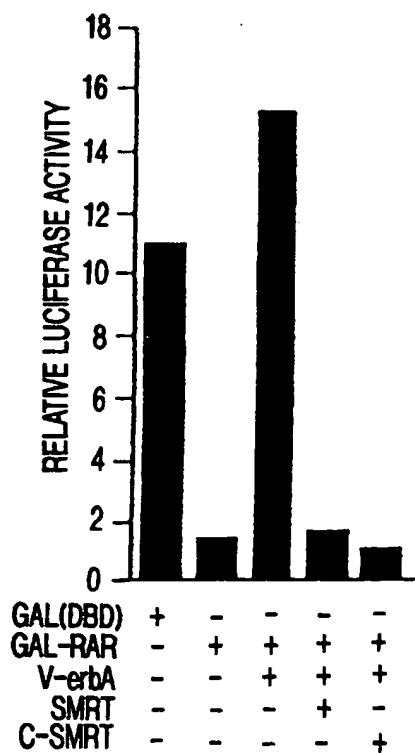


FIG. 3A

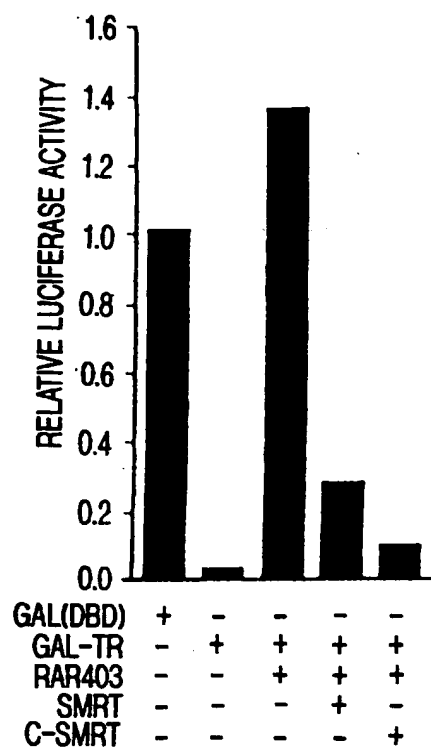


FIG. 3B

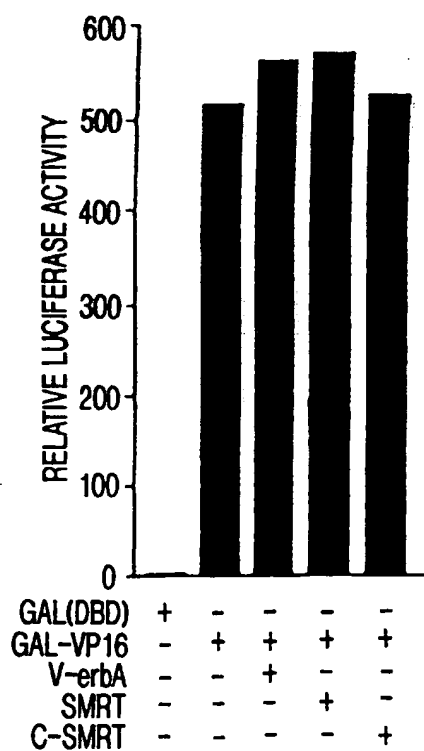


FIG. 3C

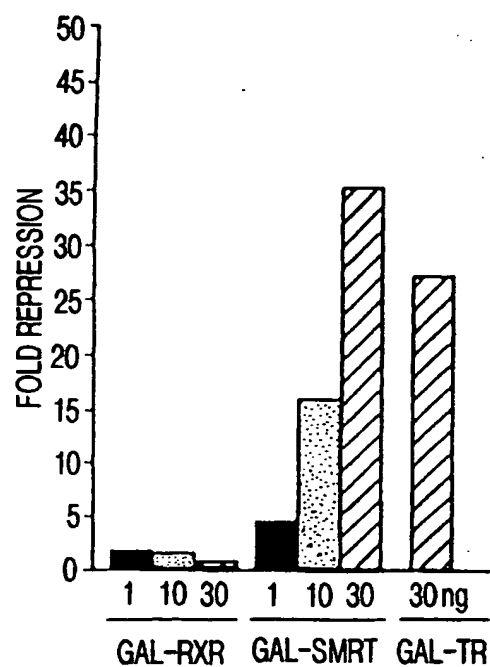


FIG. 3D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/12371

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1, 7.21, 69.1, 69.7, 240.1, 252.3, 320.1; 530/350, 300, 324, 389.2; 536/23.5, 23.1; 436/501

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Sucov et al. Retinoic acid and retinoic acid receptors in development. Molec. Neurobiol. 19 July 1995, Vol. 10, Nos. 2/3, pages 169-184, especially pages 171-181.	1-3, 6-32
Y	Leid et al. Multiplicity generates diversity in the retinoic acid signalling pathways. Trends Biochem. Sci. October 1992, Vol. 17, pages 427-433, especially pages 427-432.	1-3, 6-32
X	US 5,283,173 A (S. FIELDS) 01 February 1994, column 3-4.	1
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Y		2-3, 6-32
X	US 5,317,090 A (H.B. DE THE) 31 May 1994, columns 5-6.	1
---		---
Y		2-3, 6-32

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

29 AUGUST 1996

Date of mailing of the international search report

13 NOV 1996

Name and mailing address of the ISA/US
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12371

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C12N 1/20, 15/00, 5/00; G01N 33/53, 33/567, 33/566; C12P 21/06, 21/04; C07K 16/00, 1/00; A61K 39/395, 38/00; C07H 21/02, 21/04

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/7.1, 7.21, 69.1, 69.7, 240.1, 252.3, 320.1; 530/350, 300, 324, 389.2; 536/23.5, 23.1; 436/501

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAPLUS, MEDLINE, EMBASE, BIOSIS, JICST-EPLUS, WPIDS, PATOSEP, CONFSCI, DISSABS search terms: steroid hormone receptor, activity, suppressor, co-suppressor, binding, complex, heterodimer, homodimer, screen, assay, method

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-3 and 6-32, drawn to a co-suppressor, polynucleic acid, vector, complex comprising co-suppressor and methods of screening using co-suppressor.

Group II, claim(s) 4-5, drawn to an antibody and a method of administering said antibody.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The claims of group I share the special technical feature of a co-suppressor of steroid/thyroid hormone receptor activity whereas claims of group II do not share this special technical feature but instead share the special technical feature of an antibody and in addition each group have materially different chemical structures and materially different functional properties. These chemical structures and functional properties are the special technical features that identify each invention and distinguish each from the other because none of the special technical features is shared by the separate groups. Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.